

What is claimed is:

1. A method for detecting the occurrence or non-occurrence of an ischemic event in a patient comprising the steps of:

- 5 (a) contacting a biological sample containing albumin from said patient with an excess quantity of a metal ion salt, whereby said metal ion binds to the N-terminus of naturally occurring human albumin, to form a mixture containing bound metal ions and unbound metal ions,
- 10 (b) determining the amount of metal ions bound to the albumin N-terminus, and
- (c) correlating the amount of bound metal ions to a known value to determine the occurrence or non-occurrence of an ischemic event.

15 2. The method of claim 1, wherein said sample is serum or plasma.

3. The method of claim 1, wherein the sample is purified albumin.

20 4. The method of claim 1, wherein said metal ion salt is a salt of a metal selected from the group consisting of V, As, Co, Cu, Sb, Cr, Mo, Mn, Ba, Zn, Ni, Hg, Cd, Fe, Pb, Au and Ag.

5. The method of claim 1, wherein said metal ion is cobalt.

25 6. The method of claim 1, wherein step (b) is conducted using atomic absorption spectroscopy, atomic emission spectroscopy or an immunological assay.

7. The method of claim 6, wherein said immunological assay is conducted using an antibody specific to an antigen comprising the compound Asp-Ala-His-Lys-R, wherein R is said metal ion.

8. The method of claim 6, wherein said immunological assay is conducted using an antibody to a human serum albumin-metal complex.

9. A method of detecting the occurrence or non-occurrence of an ischemic event in a patient comprising the steps of:

5

(a) contacting a biological sample containing albumin from said patient with a predetermined excess quantity of a salt of a metal selected from the group consisting of V, As, Co, Cu, Sb, Cr, Mo, Mn, Ba, Zn, Ni, Hg, Cd, Fe, Pb, Au and Ag, to form a mixture containing metal ions bound to the N-terminus of albumin and unbound metal ions,

10

(b) contacting said mixture with an aqueous color forming compound solution to form a colored solution, wherein said compound forms color when bound to said unbound metal ion,

15

(c) determining the color intensity of said colored solution to detect the presence of unbound metal ions to provide a measure of bound metal ions, and

(d) correlating the amount of bound metal ions to a known value to determine the occurrence or non-occurrence of an ischemic event.

10. The method of claim 9, wherein said aqueous color forming compound comprises the compound Asp-Ala-His-Lys-R, wherein R is any group capable of forming color when bound to said metal ion.

20

11. The method of claim 9, wherein said sample is serum or plasma.

25

12. The method of claim 9, wherein said sample is purified albumin.

13. The method of claim 9, wherein said metal ion salt is a salt of cobalt.

14. A method for ruling-out the existence of ischemia in a patient, wherein said patient possesses one or more cardiac risk factors, comprising

- (a) application of the method of claim 1 or 9 to said patient;
- (b) subjecting said patient to an exercise treadmill test followed by a second application of the same method of claim 1 or 9;
- (b) comparing the results of the two applications of said method.

15. A method for evaluation of a patient presenting with angina or angina-like symptoms to detect the occurrence or non-occurrence of a myocardial infarction, comprising

- (a) application of the method of claim 1 or 9,
- (b) application of an electrocardiographic test,
- (c) correlating the results of step (a) with the results of said electrocardiographic test to determine the occurrence or non-occurrence of a myocardial infarction.

16. A method for supplementing electrocardiographic results to determine the occurrence or non-occurrence of an ischemic event, comprising

- (a) application of the method of claim 1 or 9,
- (b) application of an electrocardiographic test, and
- (c) correlating the results of step (a) with the results of said electrocardiographic test to determine the occurrence or non-occurrence of an ischemic event.

17. A method for comparing levels of ischemia in patients at rest and during exercise, comprising application of the following steps at designated times:

- (a) application of the method of claim 1 or 9 at a first designated time,
(b) administration of an exercise treadmill test followed by a second application of the same method employed in step (a),
(c) comparing the results of step (a) with the results obtained in step (b),
and
(d) repeating steps (a) and (b) at additional designated times,
wherein results obtained designated at each designated time are compared.

18. The method of claim 17, wherein said designated times are three months, six months and one year.

19. A method for detecting the occurrence or non-occurrence of an ischemic event in a patient comprising the steps of:

- (a) detecting the amount of endogenous copper ions present in a purified albumin sample from said patient, and
(b) correlating the quantity of copper ions present with a known value to determine the occurrence or non-occurrence of an ischemic event.

20. The method of claim 19, wherein said detecting step is conducted using atomic absorption spectroscopy, atomic emission spectroscopy or an immunological assay.

21. The method of claim 20, wherein said immunological assay is conducted using an antibody specific to an antigen comprising the compound Asp-Ala-His-Lys-R, wherein R is copper.

22. The method of claim 20, wherein said immunological assay is conducted using an antibody to a human serum albumin-copper complex.

23. A method for ruling-out the existence of ischemia in a patient, wherein said patient possesses one or more cardiac risk factors, comprising:

- 5
- (a) applying the method of claim 19 to the patient,
 - (b) subjecting said patient to an exercise treadmill test followed by a second application of the method of claim 19;
 - (b) comparing the results of the two applications of the method of claim 19.

10 24. A method for evaluation of a patient presenting with angina or angina-like symptoms to detect the occurrence or non-occurrence of a myocardial infarction, comprising

- 15
- (a) application of the method of claim 19,
 - (b) application of an electrocardiographic test,
 - (c) correlating the results of application of the method of claim 19 with the results of said electrocardiographic test to determine the occurrence or non-occurrence of a myocardial infarction.

25. A method for supplementing electrocardiographic results to determine the occurrence or non-occurrence of an ischemic event, comprising

- 20
- (a) application of the method of claim 19,
 - (b) application of an electrocardiographic test, and
 - (c) correlating the results of application of the method of claim 19 with the results of said electrocardiographic test to determine the occurrence or non-occurrence of an ischemic event.
- 25

26. A method for comparing levels of ischemia in patients at rest and during exercise, comprising application of the following steps at designated times:

- 5 (a) application of the method of claim 19 at a first designated time,
(b) administration of an exercise treadmill test followed by a second application of the method of claim 19,
(c) comparing the results of the application of the method of claim 19 prior to administration of the exercise treadmill test with the results of the application of the method of claim 19 after administration of the exercise treadmill test, and
(d) repeating steps (a) and (b) at additional designated times,
10 wherein results obtained at said designated times are compared.

27. The method of claim 26, wherein said designated times are three months, six months and one year.

15 28. A method of detecting or measuring an ischemic event in a patient comprising:

- (a) contacting a patient sample comprising naturally-occurring albumin and optionally albumin N-terminal derivatives with an excess quantity of metal ion that binds to the N-terminus of naturally-occurring albumin, whereby albumin-metal complexes are formed,
20 (b) partitioning the complexes from said derivatives, if any,
(c) measuring at least one of said derivatives, if any, and
(d) comparing said measured derivative to a known value, whereby the ischemic event may be detected or measured.

25 29. The method of claim 28 wherein said metal is Ni or Co.

30. The method of claim 28 wherein said metal of step (a) is bound to a solid support and said partitioning step (b) comprises separating said derivatives from the solid support to which the metal is bound.

31. The method of claim 28 wherein said metal of step (a) is in solution and said partitioning step (b) comprises contacting said complexes with an antibody to the albumin-metal complex, said antibody being bound to a solid support.

5 32. The method of claim 28 wherein said measuring step (c) comprises contacting said derivative with an antibody to the derivative.

33. A method for detecting or measuring an ischemic event in a patient comprising:

10 (a) contacting a patient sample comprising naturally-occurring albumin and optionally albumin N-terminal derivatives with an excess of a metal ion, whereby a albumin-metal complex is formed,

(b) contacting the mixture of step (a) with an antibody to said complex, said antibody being bound to a solid support,

15 (c) separating the complex from said N-terminal derivatives, if any,

(d) measuring the amount of at least one N-terminal derivative, if any, and

(e) comparing the measured N-terminal derivative to a known value, whereby an ischemic event may be detected or measured.

20 34. The method of claim 33, wherein the metal ion is cobalt ion.

35. The method of claim 33, wherein said measuring step (d) comprises contacting the derivative with an antibody.

25

Sub A' 5 36. An immunoassay diagnostic kit for an ischemic event comprising:
an excess quantity of a metal ion to mix with a patient sample which
comprises naturally-occurring albumin and optionally albumin N-terminal derivatives,
said naturally-occurring albumin forming a complex with said metal ion,

a first elongated solid support having a first and a second end, said first end
having a filter for application of said patient sample mixture, an area of immobilized
antibody to said albumin-metal complex between the first end the second end, and an
area of immobilized ligand to albumin proximate the second end,

10 whereby after application of said mixture of patient sample and metal ion to
said filter, said albumin-metal complex is immobilized at said area of immobilized
antibody, and said albumin N-terminal derivatives migrate and bind to the albumin
ligand proximate the second end.

15 37. The kit of claim 36, wherein said metal ion is cobalt ion.

38. The kit of claim 36, further comprising an end of process indicator at the
second end of said solid support.

20 39. The kit of claim 36, further comprising a second elongated solid support
having a first and second end, said second support first end sharing said filter for
application of said patient sample mixture with said first elongated support, and
having an area of immobilized ligand to albumin between the first and second ends,
said second support serving as a control.

25 40. The kit of claim 39, further comprising an end of process indicator at the
second end of said second solid support.

10/22/00 14:23:00

41. An immunoassay diagnostic kit for an ischemic event comprising:
a circular solid support comprising an interior filter circle surrounded by an inner concentric ring and an outer concentric ring, wherein
said inner filter circle is for application of a patient sample comprising naturally-occurring albumin and optionally albumin N-terminal derivatives, said sample having been mixed with an excess quantity of a metal ion, whereby an albumin-metal complex has been formed,
said inner concentric ring is divided into a first and second half, said first half containing a ligand to said albumin-metal complex, and
said outer concentric ring is divided into a first and second half, each said outer ring halves aligned with the inner ring halves, and each said outer ring halves containing ligands to a non N-terminus epitope of naturally-occurring albumin and to albumin N-terminal derivatives.
42. An immunoassay diagnostic kit for an ischemic event comprising:
a circular solid support comprising an inner filter circle surrounded by a concentric ring, wherein
said inner filter circle is for application of a patient sample comprising naturally-occurring albumin and optionally albumin N-terminal derivatives, said sample having been mixed with an excess quantity of a metal ion, whereby an albumin-metal complex has been formed, and
said concentric ring is divided into a first and a second half, said first half having a ligand to the albumin-metal complex, and the second half having ligands to a non N-terminus epitope of naturally-occurring albumin and to albumin N-terminal derivatives.

Sub
A2

FOR FILING

43. A method of detecting or measuring an ischemic event in a patient comprising:

- (a) contacting a patient sample comprising naturally-occurring albumin and optionally albumin N-terminal derivatives with an excess quantity of a metal ion bound to a solid support, whereby the metal ion binds to the N-terminus of naturally-occurring albumin, forming albumin-metal complexes,
- (b) separating the complexes from said derivatives, if any,
- (c) measuring at least one of said derivatives, if any, and
- (d) comparing said measured derivative to known value, whereby the ischemic event may be detected or measured.

44. The method of claim 43 wherein the metal ion is nickel ion.

45. The method of claim 43 wherein the solid support is a diacetate or a phosphonate matrix.

46. The method of claim 43 wherein said measuring step (c) comprises contacting said derivative with an antibody to the derivative.

47. A metal affinity diagnostic kit for an ischemic event comprising:
a first elongated solid support having a first and a second end, said first end having a filter for application of a patient sample, an area of immobilized metal ion between the first and the second end, and an area of immobilized ligand to naturally-occurring albumin or albumin N-terminal derivatives proximate the second end:

48. The kit of claim 47, wherein said immobilized metal is nickel.

49. The kit of claim 47, further comprising an end of process indicator at the second end of said first solid support.

50. The kit of claim 47, further comprising a second elongated solid support having a first and ~~second~~ end, said second support first end sharing said filter for application of said patient sample with said first solid support, and having an area of immobilized ligand to naturally-occurring albumin and albumin N-terminal derivatives proximate the second end, said second support serving as a control.

51. The kit of claim 50, further comprising and end of process indicator at the second end of said second solid support.

52. A monoclonal antibody directed to an epitope at the N-terminus of the albumin N-terminal derivative which lacks the four N-terminal amino acids of SEQ. ID. NO. 1.

53. A monoclonal antibody directed to an epitope at the N-terminus of the albumin N-terminal derivative which lacks the three N-terminal amino acids of SEQ. ID. NO. 1.

54. A monoclonal antibody directed to an epitope at the N-terminus of the albumin N-terminal derivative which lacks the two N-terminal amino acids of SEQ. ID. NO. 1.

55. A monoclonal antibody directed to an epitope at the N-terminus of the albumin N-terminal derivative which lacks the N-terminal amino acid of SEQ. ID NO. 1.

56. A monoclonal antibody directed to an epitope at the N-terminus of SEQ. ID NO. 2.

Sub
A4
PCT/US99/22905

57. A calibrator composition comprising a predetermined molar ratio of naturally-occurring albumin and a metal that complexes to the N-terminus of said albumin, whereby complexed albumin and unbound albumin form when said composition is in aqueous solution, wherein said ratio is between 0.1:1 and 1:0.1.

5

58. The composition of claim 57 wherein said metal is selected from the group consisting of Cu, Ni and Co.

10

59. The composition of claim ~~57~~ wherein the predetermined ratio of albumin to metal is 3:1.

60. The composition of claim ~~57~~ wherein the predetermined ratio of albumin to metal is 1:3.

15

61. The composition of claim 57 wherein the predetermined ratio of albumin to metal is 1:1.

62. A method of calibrating an analyzer that detects or measures an ischemic event according to the method of claim 1, comprising the step of:

applying the calibrator solution of claim 57 to the analyzer to determine the amount of metal ions bound to the albumin N-terminus, whereby the predetermined ratio of albumin to metal serves as a standard for calibration.

25

PCT/US99/22905

Sub 20
a⁵

5

(a) ~~mixing the calibrator composition solution of claim 57 with a predetermined amount of an excess metal salt, whereby said unbound albumin binds to said excess metal ion, generating unbound metal ions,~~

(b) ~~contacting the mixture of step (a) with color forming solution to form a colored solution,~~

(c) applying the mixture of step (b) to the analyzer, whereby the predetermined ratio of albumin to metal serves as a standard for calibration.

10

64. A method of calibrating an analyzer that detects or measures an ischemic event according to the method of claim 19, comprising the step of:

applying the calibrator solution of claim 57 wherein the metal is copper to the analyzer to determine the amount of copper ions bound to the albumin N-terminus, whereby the predetermined ratio of albumin to copper serves as a standard for calibration.

15

[illegible]

Add
96